In the Sequence Listing:

Please replace the Sequence Listing of record with the attached substitute Sequence Listing.

In the Abstract:

Page 25, line 1, please replace the heading with the following new heading:

ABSTRACT OF THE DISCLOSURE

In the Claims:

Above claim 1, insert the following:

What is claimed is:

REMARKS

The foregoing amendments are presented to place the application in compliance with the sequence rules under 37 CFR 1.821-1.825.

Applicants have submitted a revised Sequence Listing in both paper and computer readable form as required by 37 C.F.R. 1.821(c) and (e). Amendments directing its entry into the specification have also been incorporated herein. The content of the paper and computer readable copies are the same, and no new matter has been added.

The specification has been carefully reviewed and editorial changes have been effected. All of the changes are minor in nature and therefore do not require extensive discussion. Specifically,

the specification headings have been amended in conformance with U.S. practice. Further, in the section of the claims, the phrase "What is claimed is:" has also been added in accordance with U.S. practice.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

In view of the foregoing, it is believed that each requirement set forth in the Notice has been satisfied, and that the application is now in compliance with the sequence rules under 37 CFR 1.821-1.825. Accordingly, favorable examination on the merits is respectfully requested.

Respectfully submitted,

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Version with Markings to Show Changes Made

CYCLIC DEPSIPEPTIDE SYNTHETASE AND GENE THEREOF, AND MASS PRODUCTION SYSTEM FOR CYCLIC DEPSIPEPTIDE

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a cyclic depsipeptide synthetase and a gene thereof, and a mass production system for the cyclic depsipeptide. More specifically, the present invention relates to an enzyme for synthesizing substance PF1022 having anthelmintic activity and a gene thereof, and a mass production system for the substance PF1022.

2. Description of the Related Art

The substance PF1022 [cyclo(D-lactyl-L-N-methylleucyl-D-3-phenyllactyl-L-N-methylleucyl-D-lactyl-L-N-methylleucyl-D-3-phenyllactyl-L-N-methylleucyl)] is a cyclic depsipeptide which is produced by the filamentous fungus strain PF1022 (Mycelia sterilia, FERM BP-2671), which belongs to Agonomycetales, and has an extremely high anthelmintic activity against animal parasitic nematodes (Japanese Patent Application Laid-open No. 35796/1991; Sasaki, T. et al., J. Antibiotics, 45, 692, 1992). Accordingly, this substance is useful as a anthelmintic and also as a raw material for synthesizing a highly active derivative of this substance.

Generally, the amount of secondary metabolites produced by microorganisms isolated from nature is very small. Accordingly, in order to use the secondary metabolites industrially, it is necessary to improve the amount of the production. For this purpose, the culture method and the medium composition are investigated, fermentation conditions are improved by addition of precursors and the like, and strains are improved by mutation with UV irradiation or mutation inducers. Recently, in addition to these means, genetic recombination technology has become available to improve the productivity.

For example, enhancement of expression of an enzyme gene for biosynthesis, enhancement of expression of a regulatory gene for biosynthesis, and interruption of unnecessary biosynthesis pathways have been carried out (Khetan, A. and Hu, W.-S., Manual

PREFERRED EMBODIMENTS

DETAILED DESCRIPTION OF THE INVENTION

Deposition of microorganisms

The strain PF1022 described in Example 1-1 was deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry (1-3 Higashi 1-Chome, Tsukuba City, Ibaraki Prefecture, Japan), dated January 24, 1989. The accession number is FERM BP-2671.

Escherichia coli (DH5α) transformed with plasmid pPFsyn described in Example 2-1-(1) was deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry (1-3 Higashi 1-Chome, Tsukuba City, Ibaraki Prefecture, Japan), dated September 1, 1999. The accession number is FERM BP-7253.

Escherichia coli (DH5α) transformed with plasmid pPFsynN described in Example 2-1-(1) was deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry (1-3 Higashi 1-Chome, Tsukuba City, Ibaraki Prefecture, Japan), dated September 1, 1999. The accession number is FERM BP-7254.

Gene and protein

The present invention provides a cyclic depsipeptide synthetase, preferably a substance PF1022-synthesizing enzyme, and a gene thereof.

The enzyme according to the present invention acts on four molecules of L-leucine, two molecules of D-lactic acid, and two molecules of D-phenyllactic acid to synthesize substance PF1022. A derivative of substance PF1022 can be produced by in advance modifying D-lactic acid, L-leucine, and D-phenyllactic acid.

Examples of derivatives of substance PF1022 are derivatives in which two phenyl groups at the para positions in substance PF1022 are substituted by amino groups. In this case, for example, D-p-amino phenyllactic acid can be used instead of D-phenyllactic acid as a synthesizing substrate for the substance

CLAIMS

What is claimed is:

1. A protein comprising an amino acid sequence selected from the group consisting of the following sequences:

(a) an amino acid sequence of SEQ ID NO: 2, and

(b) a modified amino acid sequence of the amino acid sequence of SEQ ID NO: 2 that has one or more modifications selected from a substitution, a deletion, an addition and an insertion and has cyclic depsipeptide synthetase activity.

2. A polynucleotide encoding the protein of claim 1.

3. A polynucleotide according to claim 2, which comprises the DNA sequence of SEQ ID NO: 1.

4. A polynucleotide selected from the group consisting of the following sequences:

(c) a DNA sequence of SEQ ID NO: 1,

(d) a nucleotide sequence that has at least 70% homology to the DNA sequence of SEQ ID NO: 1 and encodes a protein having cyclic depsipeptide synthetase activity,

(e) a modified DNA sequence of the DNA sequence of SEQ ID NO: 1 that has one or more modifications selected from a substitution, a deletion, an addition and an insertion and encodes a protein having cyclic depsipeptide synthetase activity, and

(f) a nucleotide sequence that hybridizes with the DNA sequence of SEQ ID NO: 1 under stringent conditions and encodes a protein having cyclic depsipeptide synthetase activity.

5. The polynucleotide according to claim 4, wherein sequence (d) is a nucleotide sequence that has at least 80% homology to the DNA sequence of SEQ ID NO: 1.

6. The polynucleotide according to claim 4, wherein sequence (d) is a nucleotide sequence that has at least 90% homology to the DNA sequence of SEQ ID NO: 1.